SUMMARY

A new triterpene glycoside — salsoloside E, which is a bisdesmosidic glycoside — has been isolated from the epigeal part of *Salsola micranthera* Botsch.

Salsoloside E has the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-{[O-β-D-glucopyranosyl-(1 → 2)][O-β-D-xylopyranosyl-(1 → 4)]-β-D-glucuronopyranoside}.

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STEROID SAPONINS AND SAPOGENINS OF *Allium*.

XX. STRUCTURE OF KARATAVIOSIDES E AND F

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The structures of two new steroid glycosides of the spirostan series isolated from inflorescences of the plant *Allium karataviense* Rgl. (family Alliaceae) — karataviosides E and F — have been shown on the basis of chemical transformations and spectral characteristics.

We have previously reported that the inflorescences of *Allium karataviense* Rgl. (family Alliaceae) contain, in addition to karataviosides A [1], B [2], and C [3], two minor glycosides — karataviosides E and F [4]. In the present paper we present experimental information to prove the structures of the last two compounds.

The Smith degradation [5] of karataviosides E (I) and F (IV) led to the formation of karatavigenin C (II) [4].

Analysis of the product of the methanolysis of karatavioside E (I) by TLC and GLC showed that the molecule of the glycoside (I) contained D-glucose, D-galactose, and D-xylose residues in a ratio of 2:1:1. For karatavioside F (IV) using the same methods the sugars mentioned were found in a ratio of 3:1:1.

As a result of the treatment of karatavioside F (IV) with the gastric juice of the snail *Helix plectotropis* a glycoside identical with karatavioside E (I) was isolated. This shows that enzymatic hydrolysis split off one D-glucose residue.

The Hakomori methylation [6] of karatavioside F (IV) led to a permethylate (III) the IR spectrum of which did not include the band of hydroxylic absorption.

In the PMR spectrum of the permethylate (III) there were five doublets in the 4.22-5.00 ppm region corresponding to the resonance of anomeric protons of sugars. The SSCC values of the signals under discussion (^3J = 7-8 Hz) indicate the β configuration of all the glycosidic bonds [7, 8]. At the same time, it can be seen that all the carbohydrate rings are present in the C1 confirmation [9].

When the permethylate (II) was subjected to complete acid hydrolysis, a mixture of methylated sugars was isolated from the reaction mixture. After separation into individual compo-

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nents by TLC and GLC, 2,3,4,6-tetra-O-methyl-D-glucopyranose, 2,3,4-tri-O-methyl-D-xylopyranose, 4,6-di-O-methyl-D-glucopyranose, and 2,3,6-tri-O-methyl-D-galactopyranose were identified.

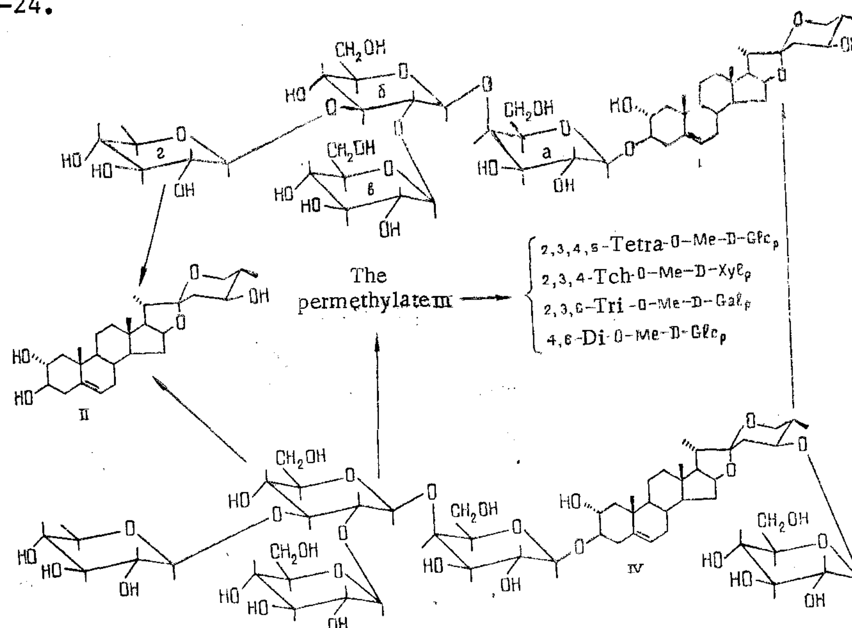
These results, considered in combination with those of the enzymatic hydrolysis of karatavioside F (IV), permit the statement that glycoside E (I) is a monodesmoside and glycoside F (IV) a bisdesmoside. These facts also indicate the identity of the tetrasaccharide residues of the two glycosides.

Further information on the position of attachment of the carbohydrate chains in compounds (I) and (IV) and on their structure was obtained by a comparative analysis of the ^{13}C NMR spectra of karataviosides E (I), F (IV), and A [1, 2] and karatavigenin C (II) [4].

In the ^{13}C NMR spectrum of karatavigenin C (II), the C-2, C-3, and C-24 carbon atoms bearing secondary hydroxy groups resonated at 72.6, 76.8, and 70.6 ppm, respectively. In the ^{13}C NMR spectrum of karatavioside E (I), the signals from C-2 and C-24 retained their positions, while, as a result of glycosylation, the signal from C-3 underwent a paramagnetic shift by +10.5 ppm and was located at 87.3 ppm.

Thus, in the molecule of glycoside E (I) the tetrasaccharide residue is bound to the aglycone through the hydroxy group at C-3 and the hydroxy functions at C-2 and C-24 have remained free.

On comparing the ^{13}C NMR spectra of karatavigenin C (II) and karatavioside F (IV) it can be seen that on passing from compound (II) to (IV) the signal from the C-2 carbon atom (76.2 ppm) retained its position, while the signals from the C-3 and C-24 underwent paramagnetic shifts by +10.5 and +10.8 ppm, respectively, and were located at 87.3 and 81.4 ppm. Consequently, the additional D-glucose residue in the pentaoside (IV) is attached to the hydroxy group at C-24.



The methylated sugars obtained by the acid hydrolysis of the permethylate (III) were identical with the products of cleavage of the permethylate of karatavioside A [1]. In view of the fact that the carbohydrate component of the glycosides isolated previously from the inflorescences of *Allium karataviense* is the tetrasaccharide lycotetraose [1-3], we are justified in assuming that the same oligosaccharide forms a component of karatavioside E (I) and F (IV).

The ^{13}C NMR spectra of karataviosides E (I) and A [1, 2] almost coincide in the region of resonance of the carbon atoms of the sugar moiety. It is sufficient to dwell on the values of the chemical shifts of the methylene carbons and the anomeric carbons (relative to TMS, ppm, solvent C_5D_5N):

Carbon atom	Karatavioside E (I)	Karatavioside A [1, 2]
C-6 a	60.6	60.6
C-6 b	62.6	62.9
C-6 c	62.6	62.9
C-6 d	67.1	66.9
C-1 a-d	103.2 104.5 104.5 104.8	103.1 104.3 104.3 104.7

The values of the chemical shifts of the carbon atoms compared are practically the same, which is possible only if the carbohydrate chains are identical.

Thus, lycotetraose is linked with the hydroxy function at C-3 in glycoside E (I) and glycoside F (IV), and in the pentaoside (IV) a D-glucose residue is attached through the hydroxy group at C-24, so that the structures of karataviosides E and F correspond to formulas (I) and (IV).

We are publishing a series of papers on the determination of the structures of spirostan and furostan glycosides and aglycones isolated from wild onions under the title "Steroid Saponins and Sapogenins of *Allium*." The first communication in this series appeared in 1970 [10]. At the same time, from the modern scientific point of view it is becoming ever clearer that the terms "steroid saponins" and "steroid glycosides" are not adequate [11]. The saponins represent the sum of the extractive substances from plants that possess surface-active properties, and they include, in addition to glycosides, other substances among which, in particular, oligosaccharides must be mentioned. Moreover, individual glycosides may not possess surface-active properties, these necessarily being individual compounds with a definite chemical structure. Consequently, the reference in the title to "steroid saponins" must be considered a tribute to a tradition of many years standing, and what is referred to is steroid glycosides of the spirostan and furostan series and their aglycones from *Allium*.

EXPERIMENTAL

General Observations. For thin-layer chromatography (TLC) we used Silufol, for prearative thin-layer chromatography type KSK silica gel (<63 μm) containing 15% of gypsum, and for column chromatography silica gel of types KSK and L (63-100 μm). Chromatography was performed in the following solvent systems: 1) chloroform—methanol—water [1a) (65:35:8); 1b) (65:30:6); 1c) (65:22:4)]; 2) butanol—ethanol—water (5:3:2); 3) chloroform—methanol (10:1); and 4) benzene—methanol (10:1).

IR spectra were taken on a UR-20 instrument in potassium bromide or paraffin oil, PMR spectra on a JNM-4H-100 (100 MHz) instrument with HMDS as internal standard, and ^{13}C NMR spectra on a Varian CFT-20 instrument (relative to TMS with C_5D_5N as solvent). Other details have been given in a previous paper [1]. The isolation of karataviosides C (I) and F (IV) has been described in [4].

Karatavioside E (I) [4], $C_{50}H_{80}L_{24}$, mp 271-275°C (from methanol, melting with decomposition), $[\alpha]_D^{25}$ −82.5 ± 2° (c 1.24; pyridine). ν_{max}^{KBr}, cm^{-1}: 845, 900, 980, 1000, 3300-3500. The methanolysis of glycoside (I) and treatment of the reaction mixture were carried out as described in [1]. D-Glucose, D-galactose, and D-xylose were detected by TLC in system 2. The ratio of the sugars according to TLC (phase I [1]) was 1.00:0.45:0.49.

Karatavioside F (IV) [4], $C_{56}H_{90}O_{29}$, melting point of crystals from methanol 252-254°C (with decomposition), $[\alpha]_D^{25}$ −73.2 ± 2° (c 1.42; pyridine), ν_{max}^{KBr}, cm^{-1}: 845, 900, 3300-3500. The condition for the methanolysis of the glycoside (IV) and the working up of the reaction mixture have been given in [1]. D-Glucose, D-galactose, and D-xylose were identified by TLC (system 2) and GLC (phase 1 [1]) in a ratio of 1.00:0.29:0.33.

Karatavioside E (I) from Karatavioside F (IV). Glycoside (IV) (300 mg) was dissolved in 100 ml of water at room temperature, and a few drops of the gastric juice of the snail *Helix plectrotropis* was added. After 16 h, the precipitate that had deposited was filtered off and dried. Separation on a column in solvent system C and recrystallization from methanol gave 180 mg of the product of the fermentation of (I): $C_{50}H_{80}O_{24}$, mp 272-274°C (decomp.), $[\alpha]_D^{25}$ −81.8 ± 2° (c 1.37; pyridine); ν_{max}^{KBr}, cm^{-1}: 845, 900, 980, 1000, 3300-3500. The fermentation product of (I) was identical with the native karatavioside E (I) according

to R_f values on TLC in systems 1a, b, and c, and according to its physicochemical constants and IR spectrum and, moreover, a mixture gave no depression of the melting point. The methanolysis of product (I) and the working up of the reaction mixture were carried out as described in [1]. D-Glucose, D-galactose, and D-xylose were detected by TLC (system 2) and GLC (phase 1 [1]) in a ratio of 1.00:0.48:0.54.

Methylation of Karatavioside F (IV). Over 1.5 h, 0.45 g of sodium hydride was added in portions to a solution of 500 mg of the glycoside (IV) in 75 ml of absolute dimethyl sulfoxide, and then the mixture was stirred for 2.5 h. After this, 8 ml of methyl iodide was added dropwise over 30 min and stirring was continued for another 3 h. All the operations were carried out at room temperature. The reaction mixture was poured into 300 ml of water and extracted with chloroform (6 × 50 ml). The chloroform extracts were combined and washed with saturated sodium thiosulfate solution (50 ml) and with water (3 × 50 ml) and were dried over anhydrous sodium sulfate. The solution was evaporated to dryness and the residue was chromatographed on a column in system 4.

This gave 500 mg of the chromatographically homogeneous permethylate (III) (TLC, system 4) the IR spectrum of which (Nujol) lacked a band of hydroxyl absorption. The permethylate (III), $C_{73}H_{124}O_{29}$ formed a white amorphous powder with $[\alpha]_D^{25}$ −41.0 ± 2° (c 1.17; chloroform); M^+ 1464. PMR spectra ($CDCl_3$, δ, ppm): 0.74, 0.91, 0.96, 1.03 (4 × CH_3, br.s), 3.10-3.30 (17 × OCH_3), 4.22, 4.29, 4.66, 4.90, 5.00 (5H, anomeric protons of sugar residues, d, ^3J = 7-8 Hz), 5.26 (H-6, br.s).

Hydrolysis of the Permethylate (III). A solution of 400 mg of compound (III) in 50 ml of methanol was treated with 45 ml of water and 5 ml of concentrated sulfuric acid and was boiled for 7 h. After this, 50 ml of water was added, the methanol was distilled off, the products of the degradation of the aglycone [4] were filtered off, and the aqueous solution was heated at ≈100°C for another 6 h. After the cooling of the reaction mixture, the acid was neutralized with ÉDÉ 10 P ion-exchange resin, the solution was evaporated to dryness, and the residue was subjected to preparative TLC in system 3. The methylated sugars obtained were identified by TLC (system 3) and GLC (phases 2 and 3 [1]):

	T_{rel}	
	Phase 2	Phase 3
2,3,4,6-Tetra-O-methyl-D-glucopyranose	1,00; 1,38	1,00; 1,32
2,3,4-Tri-O-methyl-D-xylopyranose	0,47; 0,60	0,45; 0,54
4,6-Di-O-methyl-D-glucopyranose		2,55; 2,72
2,3,6-Tri-O-methyl-D-galactopyranose	2,86; 3,48 3,74; 4,64	1,61; 2,07 2,32; 2,49

SUMMARY

Two new glycosides of the spirostan series — karataviosides E and F — have been isolated from inflorescences of the plant *Allium karataviense* Rgl.

Karatavioside E is (25S)-spirost-5-ene-2α,3β,24S-triol 3-O-{[O-β-D-glucopyranosyl-(1 → 2)][O-β-D-xylopyranosyl-(1 → 3)]-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}.

Karatavioside F has the structure of (25S)-spirost-5-ene-2α,3β,24S-triol 24-O-β-D-glucopyranoside 3-O-{[O-β-D-glucopyranosyl-(1 → 2)][O-β-D-xylopyranosyl-(1 → 3)]-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-galactopyranoside}.

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ALKALOIDS OF *Haplophyllum dauricum*

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From the roots of *Haplophyllum dauricum* (L) G. Don., collected on the territory of the Uvur-Khangai aimak, Mongolian People's Republic, in the fruit-bearing stage have been isolated robustine, dictamnine, γ-fagarine, haplopine, skimmianine, 4-methoxy-N-methyl-2-quinolone, folimine, robustinine, and the new alkaloid daurine the structure of which has been established as 8-(γ,γ-dimethylallyloxy)-4-methoxy-N-methyl-2-quinolone.

Alkaloids of plants of the genus *Haplophyllum* (family *Rutaceae*) have been described in a large number of publications [1], but many representatives of this genus growing on the territory of the SSR and outside it have not yet been investigated. These plants include, in particular, *Haplophyllum dauricum* (L) G. Don., which is found in Siberia and is widely distributed in the steppe regions of Mongolia [2]. A report in the literature [3] is limited simply to a mention of the presence of alkaloids in the Mongolian species *H. dauricum*. This plant is used in folk medicine as an antipyretic and in the treatment of some forms of tumors.

We have studied the alkaloid composition of the epigeal part and roots of *H. dauricum* collected on the territory of the Uvur-Khangai aimak, Mongolian People's Republic, in the stage of incipient fruit bearing. In an investigation of the coumarin and lignan composition of this plant in which one of us took part, skimmianine and γ-fagarine were detected [4].

The comminuted raw material was extracted with methanol. The total alkaloids were obtained by the standard method, these making up 0.75% and 0.05% of the mass of the dry roots and epigeal part, respectively. The total alkaloids from the epigeal part, containing, according to TLC, skimmianine and γ-fagarine, were not studied because of their small amount. Column chromatography of the mixture of bases from the roots of *H. dauricum* gave robustine (I), dictamnine (II), skimmianine (III), and γ-fagarine (IV). The mother liquor after the isolation of the γ-fagarine was separated into phenolic and nonphenolic fractions. Haplopine (V) was obtained from the phenolic fraction. Rechromatography of the mixed fractions followed by crystallization yielded bases with mp 117-118°C (VI) and 100-101°C (VII), folamine (VIII), and robustinine (IX).

The alkaloid (VI) is new, and we have called it daurine. As the result of a study of spectral characteristics and also the formation of 8-hydroxy-4-methoxy-N-methyl-2-quinolone on the hydrolysis of (VI) in an acid medium, the structure of 8-(γ,γ-dimethylallyloxy)-4-methoxy-N-methy-2-quinoline has been established for daurine [5]. In contrast to all known alkoxy derivatives of the 2-quinolone series, the UV spectra of which do not change on acidification and alkalinization, the spectrum of daurine undergoes a change on the addition of a drop of hydrochloric acid to an ethanolic solution of (VI) (Fig. 1). A similar change in the absorption curve in an acid medium is observed in the spectrum of 8-hydroxy-4-methoxy-N-methyl-2-quinolone (X) (Fig. 1). This gave ground for assuming that in an acid medium

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